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Continuous positive airway pressure and liver enzymes in obstructive sleep apnoea: data from a randomized controlled trial

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Abstract: **BACKGROUND:** Obstructive sleep apnoea syndrome (OSAS) has been suggested to be an independent risk factor for non-alcoholic fatty liver disease (NAFLD), possibly via intermittent hypoxia that influences blood pressure, lipid levels and insulin resistance, factors themselves known to cause NAFLD. In observational studies, OSAS has been associated with elevated levels of liver enzymes. Continuous positive airway pressure (CPAP) is the treatment for OSAS, but the effects of CPAP on liver enzymes have not been studied in a randomized controlled trial. **OBJECTIVE:** To determine if 4 weeks of CPAP influence alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) levels. **METHODS:** 94 patients with moderate-to-severe OSAS were randomized to therapeutic or sub-therapeutic CPAP treatment. Plasma ALT and AST were measured before and after 4 weeks of CPAP. **RESULTS:** Results are means \pm SD. ALT levels decreased from 39.1 ± 26.3 to 30.3 ± 16.4 IU/l in patients treated with therapeutic CPAP, but also decreased from 36.9 ± 20.7 to 31.5 ± 16.5 IU/l in patients treated with sub-therapeutic CPAP (difference between mean changes -3.4 , 95% CI -7.8 to 1.0 IU/l, $p = 0.13$ between groups). AST levels did not change significantly with therapeutic CPAP (from 29.1 ± 14.7 to 30.2 ± 13.6 IU/l), nor with sub-therapeutic CPAP (from 28.2 ± 16.2 to 29.5 ± 12.6 IU/l; difference between mean changes -0.2 , 95% CI -3.0 to 2.6 IU/l, $p = 0.87$ between groups). **CONCLUSIONS:** Four weeks of active CPAP has no beneficial effect on aminotransferase levels when compared to sub-therapeutic CPAP in patients with OSAS. Therefore, CPAP does not seem to improve biochemical markers of potential NAFLD in OSAS patients.

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Continuous Positive Airway Pressure and Liver Enzymes in Obstructive Sleep Apnoea: Data from a Randomized Controlled Trial

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Key Words

Liver transaminases • Non-alcoholic fatty liver disease • Metabolic syndrome • Obstructive sleep apnoea • Continuous positive airway pressure

Abstract

Background: Obstructive sleep apnoea syndrome (OSAS) has been suggested to be an independent risk factor for non-alcoholic fatty liver disease (NAFLD), possibly via intermittent hypoxia that influences blood pressure, lipid levels and insulin resistance, factors themselves known to cause NAFLD. In observational studies, OSAS has been associated with elevated levels of liver enzymes. Continuous positive airway pressure (CPAP) is the treatment for OSAS, but the effects of CPAP on liver enzymes have not been studied in a randomized controlled trial. **Objective:** To determine if 4 weeks of CPAP influence alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) levels. **Methods:** 94 patients with moderate-to-severe OSAS were randomized to therapeutic or sub-therapeutic CPAP treatment. Plasma ALT and AST were measured before and after 4 weeks of CPAP. **Results:** Results are means \pm SD. ALT levels decreased from 39.1 ± 26.3 to 30.3 ± 16.4 IU/l in patients treated with therapeutic CPAP, but also decreased from 36.9 ± 20.7 to 31.5 ± 16.5 IU/l in patients treated with sub-therapeutic CPAP (difference between mean changes -3.4 , 95% CI -7.8 to 1.0 IU/l, $p = 0.13$ between groups). AST levels did not

change significantly with therapeutic CPAP (from 29.1 ± 14.7 to 30.2 ± 13.6 IU/l), nor with sub-therapeutic CPAP (from 28.2 ± 16.2 to 29.5 ± 12.6 IU/l; difference between mean changes -0.2 , 95% CI -3.0 to 2.6 IU/l, $p = 0.87$ between groups). **Conclusions:** Four weeks of active CPAP has no beneficial effect on aminotransferase levels when compared to sub-therapeutic CPAP in patients with OSAS. Therefore, CPAP does not seem to improve biochemical markers of potential NAFLD in OSAS patients.

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Introduction

The obstructive sleep apnoea syndrome (OSAS) is characterized by repetitive apnoeas/hypopnoeas during sleep, which are associated with oxygen desaturation and sleep disruption. It has been estimated that between 2 and 4% of the adults in western countries suffer from clinically significant OSAS, and it is becoming more prevalent as the average body weight increases in these populations [1].

OSAS has been associated with features of the metabolic syndrome, including obesity [2], hypertension [3], dyslipidaemia [4] and insulin resistance [5], some of

Enrolment for this trial was finished before 2005.

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which are also thought to contribute to the pathogenesis of non-alcoholic fatty liver disease (NAFLD) [6, 7]. Data from recently published cross-sectional and prospective studies have implied that repetitive episodes of hypoxia occurring during sleep in patients with OSAS are an additional causal factor in the development of NAFLD, which is independent from obesity and the metabolic syndrome [8]. Furthermore, uncontrolled treatment studies of adults and children with OSAS with continuous positive airway pressure (CPAP) and tonsillectomy, respectively, were associated with a lowering of liver enzyme levels, suggesting that CPAP might improve NAFLD in patients with OSAS [9, 10].

However, there are no data from randomized controlled studies proving that CPAP treatment has a beneficial effect on liver function. To address this uncertainty, we analysed stored blood samples from a large-scale randomized controlled trial of CPAP versus sub-therapeutic CPAP in order to investigate the effects of active treatment on liver enzymes in patients with symptomatic OSAS.

Methods

Patients

Patients with possible OSAS were referred to the Oxford Sleep Unit, Oxford Centre for Respiratory Medicine, UK by general practitioners, ear, nose and throat surgeons or other hospital consultants. Patients were eligible for the trial if they were males aged between 20 and 75 years who had excessive daytime sleepiness (Epworth Sleepiness Scale score ≥ 10) and proven OSAS with >10 oxygen desaturations of $>4\%$ per hour (oxygen desaturation index, ODI, $>10/h$). All eligible patients were offered participation in the study, unless they required urgent CPAP therapy because of respiratory failure, driving or employment issues. None of the patients had a medical history of a liver disease. The study was approved by the Oxford research ethics committee, and written informed consent was obtained from all participants. Data on blood pressure from 52 of these patients had been presented in a previously published study evaluating the effect of CPAP on ambulatory blood pressure [3].

Sleep Study, CPAP and Assessment of Sleepiness

OSAS was diagnosed from 1-night, in-hospital, pulse oximetry and ECG, combined with continuous video recordings. Patients' body movements, heart rates and pulse transit time changes were recorded as measures of arousal from sleep. Pulse oximetry, snoring and increases in the respiratory swing in pulse transit time were used as markers of breathing pattern and respiratory effort (Win-Visi monitoring system, Stowood Scientific Instruments, Oxford, UK) as previously described and validated [11, 12]. The results of the sleep study were scored automatically, with manual review to ensure accuracy of the data. OSAS was diagnosed from a review of all the data and a clinical interview, and

the severity was quantified as the number of oxygen desaturations $>4\%$ per hour of study (the ODI).

After enrolment, patients were randomly assigned to either therapeutic or sub-therapeutic CPAP, and they then underwent a second sleep study, during which respiratory polysomnography was repeated and CPAP was used according to the assigned group. For patients assigned to therapeutic CPAP, the therapeutic pressure was determined from overnight use of the Sullivan AutoSet-T auto-adjusting CPAP machine (ResMed, Abingdon, UK), from which mask pressure was recorded and synchronized with the sleep study signals. The record was reviewed the next morning, and the optimum pressure to prevent sleep apnoea, usually the 95th percentile of pressure overnight, was confirmed by a sleep technician. Patients assigned to sub-therapeutic CPAP used a machine that delivered <1 cm H₂O pressure, as previously described [3], which is insufficient to hold the pharynx open [13].

Patients were blind to whether they were receiving therapeutic or sub-therapeutic CPAP, and so were the investigators. The sleep nurse who randomly assigned patients to the 2 groups, maintained the machines and assisted the patients was not involved in outcome assessments.

Subjective sleepiness was assessed using the Epworth Sleepiness Scale, which assesses the tendency to fall asleep during 8 typical daytime situations [14]. Objective sleepiness was measured with 1 sleep resistance challenge (Osler test), which tests the ability to stay awake in a darkened and sound isolated room. This was carried out at the same time of day on the 2 occasions each patient was studied [15].

Liver Enzymes

Measurements of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed on plasma samples which were stored at -80°C . ALT and AST were measured in pairs of baseline/follow-up samples, using a standard automated enzymatic assay (ADVIA 2400, Siemens, Frimley Park, UK) as previously described [16, 17]. Method precision, expressed as the % coefficient of variation, was 3.6% at 30 IU/l and 2.5% at 140 IU/l for AST, and 3.9% at 42 IU/l and 2.8% at 140 IU/l for ALT.

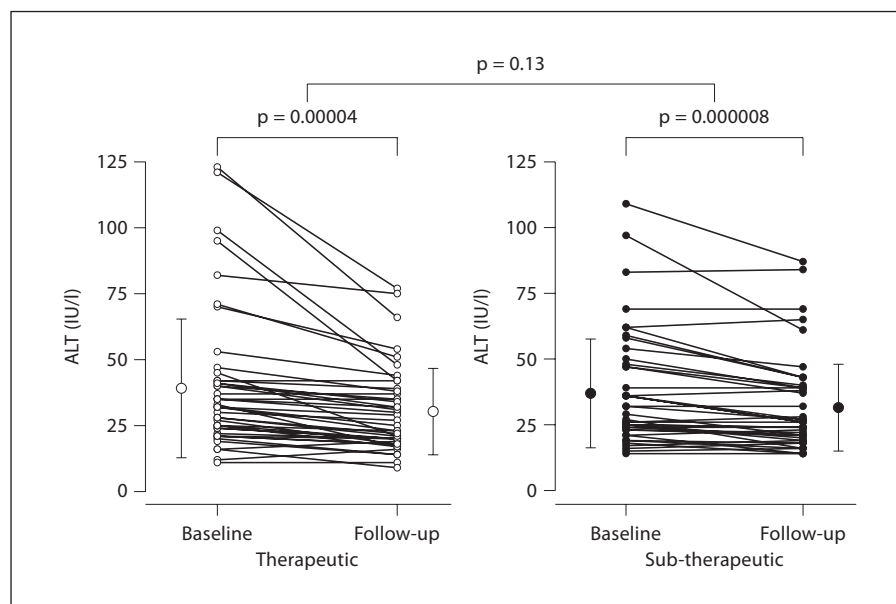
Follow-Up

After baseline assessments, patients used their therapeutic or sub-therapeutic CPAP machine (Sullivan 6, ResMed, Abingdon, UK) for 4 weeks and then returned to the treatment centre for repeat measurements. Hour meters on the CPAP machines were downloaded to calculate mean nightly use. At the end of the trial, CPAP pressure was retitrated in every patient to establish their therapeutic pressure for subsequent long-term use.

Data Analysis

Data are expressed as mean \pm SD unless otherwise stated in the text. All statistical analyses were performed with Statistica v6.0 (StatSoft, Tulsa, Okla., USA). Differences between and within the groups at baseline and after 4 weeks were assessed by unpaired and paired *t* tests as appropriate. Differences between the changes seen in each group are expressed as the mean and 95% CIs, therapeutic minus sub-therapeutic. Data were analysed on an intention-to-treat basis, with no change assumed when follow-up liver enzyme data were missing. Data were also analysed as per the protocol (with no data assumption, thus including only those

Fig. 1. Individual ALT levels at baseline and follow-up in the therapeutic CPAP group (left panel) and sub-therapeutic CPAP group (right panel). Large circles and vertical bars are means \pm SD. The mean ALT levels decreased significantly after 4 weeks of therapeutic and sub-therapeutic CPAP, but there was no statistical difference in the change between the therapeutic and sub-therapeutic CPAP groups.



subjects with complete follow-up data) to assess whether this differed from the intention-to-treat analysis. For comparison of frequencies, the χ^2 test of independence was used. Spearman's rank test was used for correlation analysis. $p < 0.05$ was considered to be statistically significant.

Results

Patients Characteristics

Of the 102 patients in the original trial, 94 patients with a mean age of 48.3 ± 9.9 years had stored blood samples. Of these patients, 47 were randomized to therapeutic and 47 to sub-therapeutic CPAP. The 2 groups were similar regarding age, body mass index (BMI), fat distribution, alcohol consumption, smoking status, frequency of hypertension (and antihypertensive medication) and severity of sleep apnoea (table 1).

Liver Enzymes

Blood samples from 94 patients were available for analysis at baseline, and from 87 at follow-up (44 in the therapeutic and 43 in the sub-therapeutic group). ALT levels decreased from 39.1 ± 26.3 to 30.3 ± 16.4 IU/l in patients treated with therapeutic CPAP for 4 weeks, but they also decreased from 36.9 ± 20.7 to 31.5 ± 16.5 IU/l in patients treated with sub-therapeutic CPAP (difference between mean changes -3.4 , 95% CI -7.8 to 1.0 IU/l, $p = 0.13$ between groups). The blood levels of AST did not

change significantly with therapeutic CPAP (from 29.1 ± 14.7 to 30.2 ± 13.6 IU/l), nor with sub-therapeutic CPAP (from 28.2 ± 16.2 to 29.5 ± 12.6 IU/l; difference between mean changes -0.2 , 95% CI -3.0 to 2.6 IU/l, $p = 0.87$ between groups). Individual changes in ALT are shown in figure 1, and changes in AST are shown in figure 2.

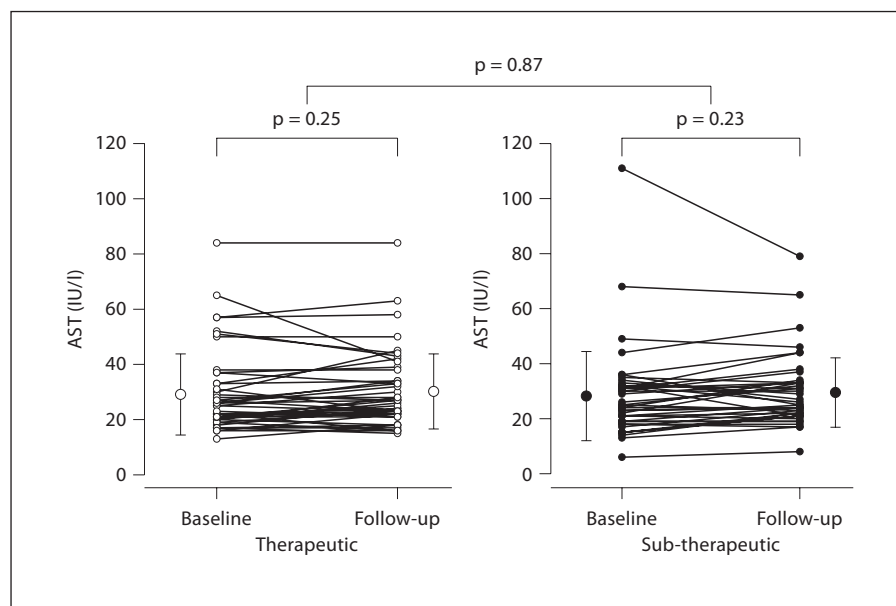
When data on ALT and AST were analysed as per the protocol, again there was no statistically significant difference in changes between the therapeutic and sub-therapeutic CPAP groups.

There was no statistically significant correlation between ALT levels and ODI ($r = 0.17$, $p = 0.10$), BMI ($r = 0.15$, $p = 0.16$) nor between AST levels and ODI ($r = 0.16$, $p = 0.13$) and BMI ($r = 0.13$, $p = 0.20$).

Measures of Sleepiness

Therapeutic CPAP significantly reduced the Epworth Sleepiness Scale score compared to the sub-therapeutic CPAP group: therapeutic 15.8 ± 4.2 to 6.8 ± 5.1 and sub-therapeutic 15.2 ± 3.8 to 11.6 ± 5.9 (mean difference between groups -4.7 , 95% CI -6.8 to -2.6 , $p < 0.0001$). Therapeutic CPAP also significantly improved objective sleepiness measured by the Osler test compared to sub-therapeutic CPAP: therapeutic 17.9 ± 13.0 to 26.2 ± 12.7 min and sub-therapeutic 17.5 ± 12.9 to 18.5 ± 14.2 min (mean difference between groups 7.0 , 95% CI 1.1 to 13.0 min, $p = 0.019$). Compliance with CPAP did not differ between the 2 groups (table 1).

Fig. 2. Individual AST levels at baseline and follow-up in the therapeutic CPAP group (left panel) and sub-therapeutic CPAP group (right panel). Large circles and vertical bars are means \pm SD. The mean AST levels did not change significantly after 4 weeks of therapeutic and sub-therapeutic CPAP, and there was no statistical difference in the change between the therapeutic and sub-therapeutic CPAP groups.



Discussion

We present the first data from a randomized controlled trial investigating the effects of CPAP on liver enzyme levels in patients with moderate-to-severe OSAS. We found that blood aminotransferase levels decreased in the group treated for 4 weeks with therapeutic CPAP and in the group receiving sub-therapeutic CPAP. This decrease in liver enzymes was not different between the 2 groups, despite clear evidence of a differential effect of CPAP, with a clear improvement in both subjective and objective sleepiness in patients treated with therapeutic CPAP compared with sub-therapeutic CPAP.

However, the majority of subjects had liver enzyme levels within the normal range (77%), and therefore improvements might not have been expected. We therefore analysed data from patients with ALT levels above and below the upper limit of the normal range separately (which is 45 IU/l at our institution) and found, again, no significant differences in changes between the therapeutic and sub-therapeutic groups. In addition, in patients with ALT values in the normal range (<45 IU/l), levels also decreased significantly in both the therapeutic and sub-therapeutic groups, indicating that a potential floor effect was not a confounder.

Obesity, insulin resistance and dyslipidaemia have been shown to contribute to the development of NAFLD [6, 7]. According to a hypothesis Day and Saksena published in 2002 [18], increased oxidative stress may be an

Table 1. Patient characteristics

	Sub-therapeutic CPAP (n = 47)	Therapeutic CPAP (n = 47)	p value
Age, years	48.5 \pm 10.4	48.0 \pm 9.5	0.81
Weight, kg	110.2 \pm 19.2	115.0 \pm 24.5	0.30
BMI	34.4 \pm 4.6	35.6 \pm 7.1	0.33
Neck circumference, cm	44.6 \pm 3.0	45.1 \pm 3.9	0.55
Waist/hip circumference ratio	1.00 \pm 0.05	1.01 \pm 0.06	0.60
Alcohol intake, g/day	6.3 \pm 7.9	9.0 \pm 13.3	0.23
Current smoker, %	19.2	21.3	0.80
Ex-smoker, %	53.2	44.7	0.41
Hypertensive, %	25.5	21.3	0.63
Diabetic, %	0.0	2.1	0.32
Oxygen saturation dips $>4\%$, n/h of sleep	44.4 \pm 21.5	40.4 \pm 25.0	0.41
Epworth Sleepiness Scale score at baseline	15.2 \pm 3.8	15.8 \pm 4.2	0.51
Osler at baseline, min	17.5 \pm 12.9	17.9 \pm 13.0	0.87
CPAP compliance, h/night	3.9 \pm 2.5	4.6 \pm 2.1	0.18
Retitrated CPAP pressure following study, cm H ₂ O	10.2 \pm 1.7	9.9 \pm 1.8	0.51

Values are means \pm SD or simple percentages.

additional independent factor which may lead to hepatocellular injury and thereby promote the development of NAFLD. As OSAS has been associated with insulin resistance [5], dyslipidaemia [4] and increased oxidative stress [19], it seems reasonable to assume that OSAS might

be an independent risk factor for the development of NAFLD. Consistent with this hypothesis, several observational studies have suggested a link between OSAS and NAFLD, having shown a correlation between markers of hypoxia, sleep disordered breathing and blood markers of liver function in patients with OSAS [8, 20, 21]. Interpretation of these associations has been difficult, since 2 of these studies had either no or an inadequate control group [8, 21]. Additionally, in the study by Tatsumi and Saibara [20], only serum levels of type III procollagen (a marker of latent steatohepatitis), but not aminotransferases, were associated with average SaO₂ during sleep. Furthermore, ALT and AST levels were not different in patients with OSAS, compared with well-matched control subjects without OSAS in the latter study.

Only 2 studies have investigated the effect of OSAS treatment on serum aminotransferase levels [9, 10]. In an uncontrolled study including 40 patients with moderate-to-severe OSAS, Chin et al. [9] found a decrease in AST of 5 IU/l after a single night of CPAP treatment, but surprisingly CPAP had no effect on the more sensitive ALT; this effect persisted at the 6-month follow-up. Kheirandish-Gozal et al. [10] recently reported a higher prevalence of OSAS among obese children with 'NAFLD' (which they defined entirely by elevated serum aminotransferases) compared to obese children without 'NAFLD'. In the same study, uncontrolled treatment of OSAS with tonsillectomy (and CPAP) improved liver enzymes in 76% of children.

In contrast to these reports, the findings of our study show that CPAP treatment has no beneficial effect on liver enzymes when compared to a sham treatment. The decrease in ALT we observed in both the therapeutic and the sub-therapeutic CPAP groups seems, therefore, to be an effect independent of OSAS treatment. A possible explanation for the improved ALT levels may be a non-specific placebo effect, or the well-known fact that patients alter their behaviour when they enter a study (e.g. they are more compliant with a prescribed medication or follow a healthier lifestyle), which may bias the outcome in an un-

controlled study [22, 23]. It is possible that patients in our study also changed their lifestyle during the trial (e.g. reduced alcohol consumption, avoided foods rich in calories or fat, began to exercise), which may explain the decreased levels of ALT that we found after 4 weeks of both therapeutic and sub-therapeutic CPAP. That only ALT decreased and not AST may be explained by the fact that ALT is more specific for liver disease (especially NAFLD) [24, 25] and falls in response to improvements in lifestyle [26].

At this point it must be mentioned that the gold standard for the diagnosis of NAFLD would be a liver biopsy. As liver enzymes are not specific markers of liver function in NAFLD, it may be difficult to investigate the true relationship between OSAS and NAFLD without liver biopsy. Therefore, further randomized controlled studies including carefully selected patients with histologically proven NAFLD and OSAS would be needed to accurately define this potential relationship.

In conclusion, this double-blind randomized controlled trial has shown no extra improvement in liver function (measured by aminotransferases) in patients with symptomatic OSAS following active CPAP treatment for 1 month, when compared to sub-therapeutic CPAP, despite a clear differential improvement in both subjective and objective sleepiness.

The reasons for this similar improvement in both groups are not clear, but may be due to the fact that patients behave differently when entering a clinical trial and thereby producing non-specific effects. Therefore, CPAP does not seem to improve liver function in a typical cohort of OSAS patients.

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